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New records of *Tripedalia cystophora* Conant, 1897 (Cubozoa, Carybdeida) along the southeastern Florida coastline

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Abstract. Regional changes in coastal climates have recently resulted in many marine species expanding their natural ranges poleward. Among these newly migrating species is the Mangrove Box Jelly, *Tripedalia cystophora* Conant, 1897 (Cubozoa, Carybdeida). Discovery of this tropical species on the western coast of Florida has been well documented, but records on the eastern coast of Florida are less definitive. We identified 99 individuals in southeast Florida, including adults of both sexes. Evidence of a stable, breeding population of this box jelly on the southeast Florida coast was indicated.

Keywords. Cubozoan, geographic distribution, Caribbean, range expansion

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Introduction

The recent shifting and expansion of marine species populations towards polar regions have been reported worldwide (Cheung et al. 2012; Nakamura et al. 2013; Ekins and Gershwin 2014; Pacifici et al. 2015; Tuckett et al. 2017). As sea surface temperatures warm, new marine habitats become available that were previously unsuitable (Byrne et al. 2011; Reddin et al. 2018, 2020; Kingsbury et al. 2020). This is particularly well observed for tropical Caribbean species that have moved north into the Gulf of Mexico (Ahuatzin-Hernández et al. 2019), and/or subtropical and temperate species up the eastern seaboard of the United States (Nye et al. 2009).

The Mangrove Box Jelly, *Tripedalia cystophora* Conant, 1897, has recently immigrated into Florida waters from the Caribbean Sea (Orellana and Collins 2011; Lasley et al. 2016). Lasley et al. (2016) first recorded *T. cystophora* in 2007 on the Gulf coast of Florida in Tampa Bay, with subsequent discoveries through 2015 along much of the southwestern Florida coastline area southward. Along the Atlantic eastern side of Florida within Lake Wyman, Boca Raton, Intracoastal Waterway, *T. cystophora* was first reported in 2009 by Orellana and Collins (2011), who identified a single male individual with an unknown population status. Orellana and Collins (2011) suspected it to be more prevalent, but only formally noted one individual. The species was recently added to the John D. MacArthur Beach State Park Unit Management Plan species list in 2020 (FLDEP 2020), but no information was available on numbers or notation. Through subsequent observations, we examined

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several sites in Palm Beach and Martin Counties to determine if a persistent natural population of *T. cystophora* was present in southeast Florida. We also noted the current northern extent of the species along the eastern coastline of Florida.

Methods

Live specimens of *Tripedalia cystophora* were observed, captured, and collected during 13 sampling periods from July to October 2021. Individuals were found at six mangrove locations along the Intracoastal Waterway and Lake Worth Cove (Fig. 1). Southernmost sites were located at John D. MacArthur Beach State Park, North Palm Beach, Palm Beach County, FL, USA to the northern extent adjacent to Jonathan Dickinson State Park, Hobe Sound, Martin County, FL, USA. Additional anecdotal observations from June to July 2017 and June to July 2018 taken at John D. MacArthur Beach State Park (MBSP) were also noted. In July and September 2022, four additional northern sites along the intracoastal waterway were sampled. Those sites ranged from the intracoastal waters adjacent to the Nathaniel P. Reed Hobe Sound National Wildlife Refuge, Hobe Sound, FL, USA to Stuart Causeway Beach, Stuart, FL, USA.

Specimens were collected by varied methods including cast net (9.525 mm mesh), seine net (4 mm mesh), plastic zipper bags, and visual observations (Table 1). Using plastic zipper bags, six animals were captured during the October 2021 sampling at John D. MacArthur Beach State Park and retained for further examination (Florida Department of Environmental Protection Scientific Research/Collecting Permit no. 09102115). These specimens were maintained as vouchers in the Department of Biology, Palm Beach Atlantic University, West Palm Beach, FL, USA, Invertebrate Collection. Collected specimens were preserved in formalin, diluted to 10% with native seawater.

Identifications were completed via morphological characteristics, using descriptions, identifications, and imagery in available literature (Bentlage and Lewis 2012; Straehler-Pohl et al 2014; Lasley et al. 2016; Helmark and Garm 2019). Sex determination was completed using microscopy and comparing to current available literature (Helmark and Garm 2019). Systematics was confirmed by consulting WoRMs (2023).

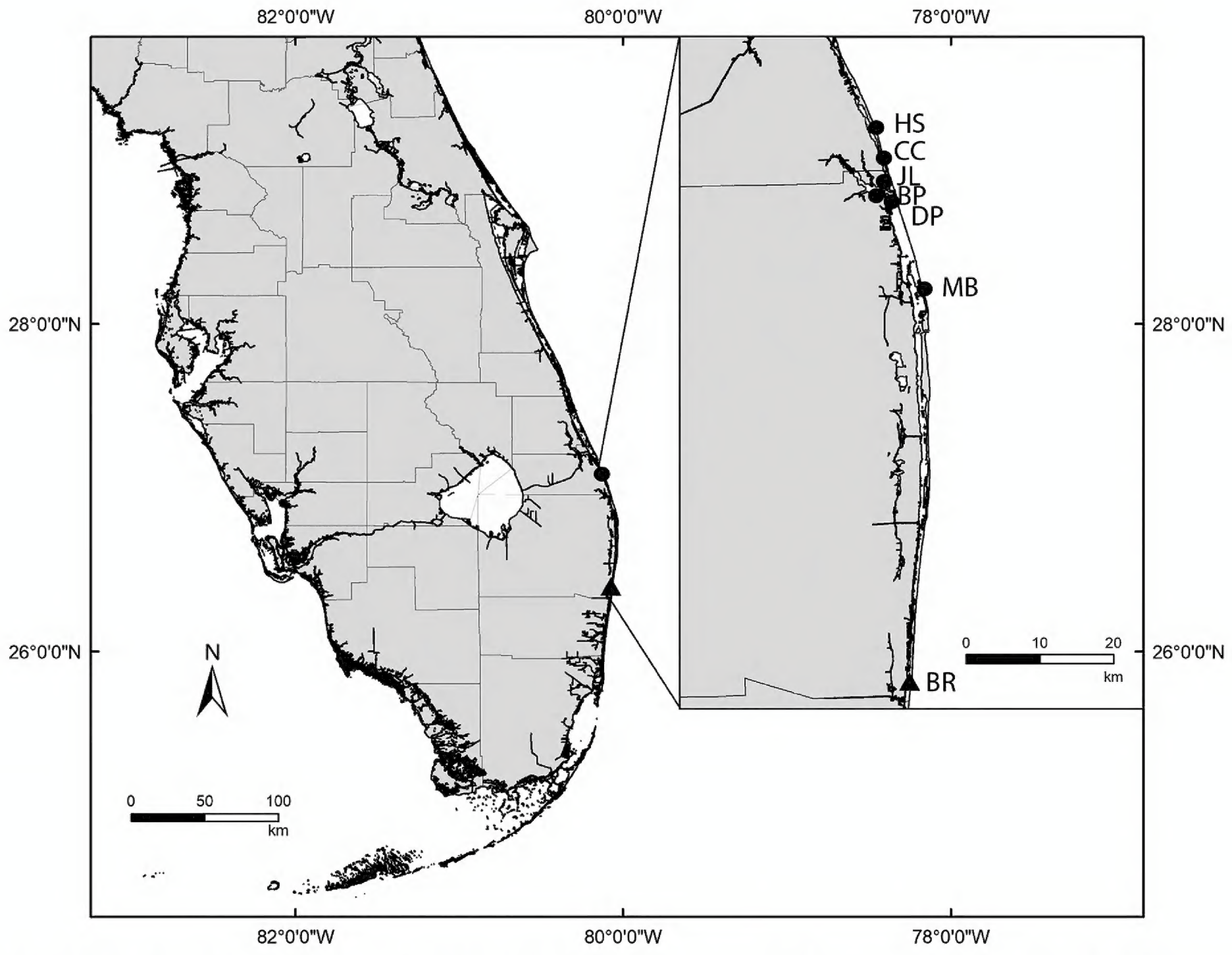

Figure 1. Florida map of *Tripedalia cystophora* survey sites. Solid circle symbols indicate sampling sites from this study: HS = south of Hobe Sound, adjacent to Johnathan Dickinson State Park; CC = Coral Cove, Tequesta, FL; JL = Jupiter Lighthouse, Jupiter, FL; BP = Burt Reynold Park, Jupiter FL; DP = Dubois Park, Jupiter, FL; MB = John D. MacArthur State Park, North Palm Beach, FL. Triangle symbol indicates sampling site from the previous record on the Atlantic coast: BR = Lake Wyman, Boca Raton, FL (Orellana and Collins 2011).

Table 1. Listing of all individual *Tripedalia cystophora* sampled in 2017–2021 and their method of collection. All specimens were found on the southeast coast of Florida within the Intracoastal Waterway near/among mangroves or above seagrasses. HS = south of Hobe Sound, adjacent to Johnathan Dickinson State Park; CC = Coral Cove, Tequesta, FL; JL = Jupiter Lighthouse, Jupiter, FL; BP = Burt Reynold Park, Jupiter FL; DP = Dubois Park, Jupiter, FL; MB = John **D.** MacArthur State Park, North Palm Beach, FL. * indicates sampling where specimens were retained in collection.

Date	No. of specimens	Method of collection	Location
VI–VII.2017	20	Visual count	MB
VI–VII.2018	10	Visual count	MB
21.VII.2021	12	Seine net	BP
22.VII.2021	16	Cast net	BP
24.VII.2021	8	Cast net	BP
28.VII.2021	1	Visual count	HS
29.VII.2021	3	Visual count	JL
2.VIII.2021	9	Visual count, zipper bag*	MB
19.VIII.2021	3	Cast net	CC
5.X.2021	2	Visual count, zipper bag*	MB
5.X.2021	4	Visual count, zipper bag*	MB
7.X.2021	6	Seine net	HS
25.X.2021	5	Cast net	DP

Results

Class Cubozoa Werner, 1973
Order Carybdeida Gegenbaur, 1857
Family Tripedaliidae Conant, 1897

Tripedalia cystophora Conant, 1897

Figure 2

New Records. UNITED STATES OF AMERICA – Florida • Palm Beach and Martin Counties, Lake Worth Lagoon, John D. MacArthur Beach State Park-Hobe Sound; 26°49′30″N, 080°02′33″W – 27°00′02″N, 080°05′30″W; 0.5–1.5 m depth; V–VI.2017 to 5.X.2021; Alaina Bell, Zachary M. Miller, Angela D. Witmer obs. & leg.; 99 individuals, includes 6 collected; IC2021-01, IC2021-02.

Identification. *Tripedalia cystophora* is a small box jelly, with a bell width of less than 14 mm (Lasley et al. 2016; Ahuatzin-Hernández et al. 2019). It has a cube-shaped bell with 4 recognized corners, a lightly arched apex, and sparsely, clustered nematocysts on the bell (Straehler-Pohl et al. 2014). Each corner has 3 pedalia extending from the bell margin with each pedalia having one tentacle. Rhopalia are located on each side of the bell approximately ⅓ up from the bottom margin. The rhopalium lies inside a niche ostium creating a distinct hooded appearance. Male individuals were identified by their densely packed hemi-gonads with sperm and/or the presence of a spermatozeugma (Helmark and Garm 2019). Female individuals were identified by presence of fertilized eggs, zygotes, or planula within the hemi-gonads or gastric pouches (Helmark and Garm 2019). Female hemi-gonads were also long, extending nearly the entire length of the bell (Stewart 1996). If eggs, dense sperm, or spermatozeugma were not identified, the individual was labeled as an immature specimen. Individuals identified in Florida did not appear to be morphologically different from Caribbean individuals as described in the literature.

Remarks. During this study 99 individual mangrove box jellies were observed from 2017–2021 (Table 1). Sixty-nine individuals were observed during 2021. From these 69, six individuals were collected and retained for voucher use. Of the six voucher specimens, four were identified as female, one as male (Fig. 2A), and one as a sexually immature individual. The largest female identified was gravid with eggs inside the gastric pouches (Fig. 2B). Bell widths of the preserved vouchers ranged from 7–12 mm with the largest being the gravid female. Immature individual was measured: 8×7 mm (length by width). Male was measured at 7×7 mm (length by width). Females were measured: 8×7 mm, 8×7 mm, 9×8 mm, 10×12 mm (length by width). Typical species bell length by widths include: 10.9×12.8 mm (Ekins and Gershwin 2014) and 8.6×10.3 mm (Ahuatzin-Hernández et al. 2019).

Individuals were observed in northern Palm Beach County up to the middle of Martin County within the Lake Worth Lagoon system/Intracoastal Waterway. This study extended the known range of *T. cystophora* 72 km (45 mi.) north along the southeast Florida coastline. In 2022, four favorable locations further north in the Intracoastal Waterway into the Indian River Lagoon were examined for possible *T. cystophora*. No individuals were found during those surveys.

Discussion

Previous record of this species on the Atlantic Florida coastline was limited to a single confirmed animal and additional anecdotal reports (Orellana and Collins 2011). It was not known if this was a stable reproducing population or just a random individual swept in by Caribbean currents (Orellana and Collins 2011). Our discovery and examination of numerous individuals at multiple locations north of the original 2009 report in Boca Raton waters confirms that there is a stable population of *Tripedalia cystophora* along the southeast Florida coastline in northern Palm Beach County and Martin County. The identification of a gravid female and numerous immatures further confirms a breeding population. Unlike most cnidarians, *T. cystophora* practices internal fertilization via courtship behaviors, mating, and the male transfer of sperm packets (spermatozeugma) into the female (Bentlag and Lewis 2012; Helmark and Garm 2019). Thus, a sustainable breeding population for this species would require both male and female individuals to be both common and easily accessible to one another. We were able to identify both males and females of the species along with varied

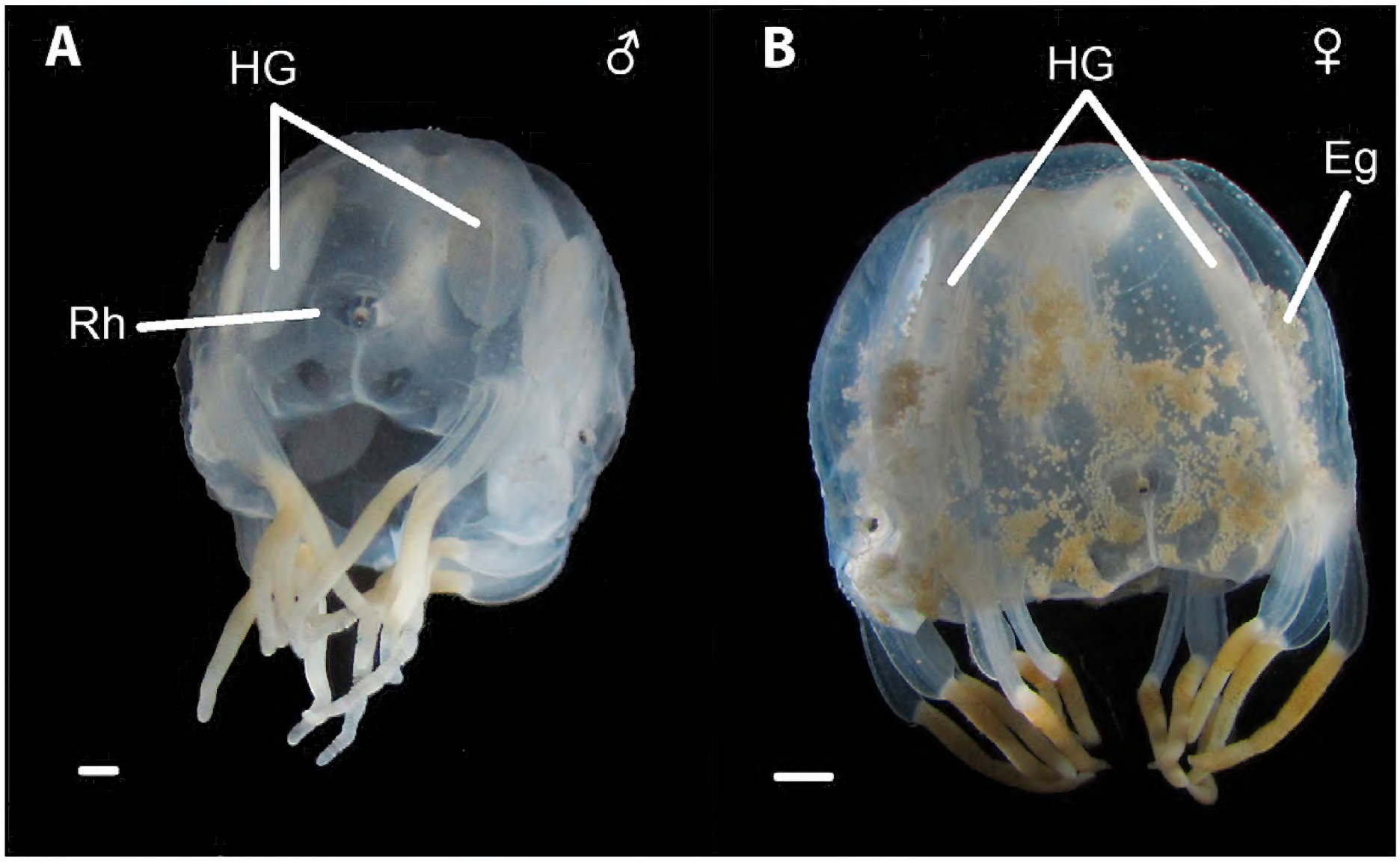

Figure 2. Photographs of preserved *Tripedalia cystophora* in 10% formalin. **A.** sexually mature male, measuring 7×7 mm (width×height). Hemi-gonads (HG) are indicated, densely packed with sperm. Rh = rhopalium. **B.** gravid female, 12 mm×10mm (width×height). Hemi-gonads (HG) are spent with eggs (Eg) outside in bell chamber, being released. Scale bars: 1 mm.

medusa ages from immature to young female to gravid female.

Using as a baseline Orellana and Collins' (2011) first report of *T. cystophora* along the Florida Atlantic coastline in 2009, we observe that the Mangrove Box Jelly has now been identified 72 km north in 13 years. This exceeds the decadal migration rates of other marine species experiencing poleward latitudinal range shifts (Sorte et al. 2010; Cheung et al. 2012; Jones et al. 2013). This species quite possibly migrated from tropical mangrove locations to Florida via the Caribbean current to the Loop Current (which may feed populations on the western coast of Florida) then through the Florida Current to the southeastern Florida coastline. Movement via the Loop Current and Florida Current has been documented in several marine species (Foley et al. 2013; Chaves-Fonnegra et al. 2015; Bertola et al. 2020). Lasley et al. (2016) speculated that *T. cystophora*'s initial discovery on Florida's west coast may not be natural but the results of human introduction. While it is possible for Caribbean individuals to replenish or supplement the now perceived local population, the varied stages of medusa individuals found from immature to gravid female indicates a local breeding population. Favorable temperatures for development have been found to range between 29 and 31 °C (Werner et al. 1971; Buskey 2003; Helmak and Garm 2019). Temperatures within Lake Work Lagoon has been recorded at those favorable temperatures during the months of June to October annually (LWL-4 station) (SFWMD n.d.). As favorable temperatures for this species continue to increase northward along the Atlantic Florida coastline, we expect that *T. cystophora* may very well populate itself further up the Florida peninsula in northern mangrove habitats. A review of species and management plan from local agencies and intracoastal state parks north of our study sites in the Indian River Lagoon have found that this species has yet to be reported (SMS n.d.; FLDEP 2014).

Biogeographic shifts brought about by changes in climate are neither new nor poorly studied phenomena (Reddin et al. 2018, 2020). Non-local but adjacent ecosystem species are known to migrate poleward from low to high latitudes as the latter habitats' climates become more favorable to them. In some cases, immigration of non-native species into new habitats leads to significant changes in biodiversity (Cheung et al. 2012; Dornelas et al. 2014; Woodworth-Jefcoats et al. 2017; Kingsbury et al. 2020). It has also been noted that displacement of native species and/or niche partitioning by the immigrating species may result (Jones et al. 2013; Nakamura et al. 2013; Pacifici et al. 2015; Woodworth-Jefcoats et al. 2017). For *T. cystophora*, we have observed in the past decade the movement of this species from tropical mangrove systems to subtropical mangrove systems. It is recommended that further investigation continue on the species' northern migration into eastern Florida waters. Further research into how this tropical cubozoan has inserted itself into local food webs, how abundant it has become, and how may be altering native ecosystem structure are all important questions.

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Author Contributions

Conceptualization: ADW, TC. Data curation: ACB, ADW, ZM, ST. Formal analysis: ADW, TC. Methodology: ADW, ZM. Project administration: ADW. Resources: ST. Writing – original draft: ADW. Writing – review and editing: TC.

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